WHEAT GERM SUPPLEMENT REDUCES CYST AND TROPHOZOITE PASSAGE IN PEOPLE WITH GIARDIASIS

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Abstract. The protozoan parasite Giardia lamblia is a major cause of waterborne enteric disease worldwide. Lectins are proteins that bind to carbohydrate (sugar) moieties. Potential targets for lectins are found on the surface of most single-celled organisms. Modest concentrations of wheat germ agglutinin (WGA) have been shown to inhibit G. lamblia excystation and trophozoite growth in vitro and can reduce cyst passage in mice infected with the closely related protozoan parasite, G. muris. Commercial preparations of wheat germ (WG) contain 13–53 µg of WGA per gram. We performed a double-masked, placebo-controlled study of dietary supplementation with WG in 63 subjects with giardiasis in Montreal and Lima (25 asymptomatic patients passing cysts; 38 patients with symptoms). Asymptomatic subjects received WG (2 g, 3 times a day) or placebo (cornstarch, 2 g, 3 times a day) for 10 days, followed by metronidazole (250 mg 3 times a day) for 7 days. Symptomatic subjects received metronidazole (250 mg 3 times a day) plus either WG or placebo for 7 days. Stool specimens were collected every day (Montreal) or every other day (Lima) for 10 days and on Day 35 for microscopic examination and coproantigen determination. Subjects kept a diary of symptoms for 10 days after treatment. In asymptomatic subjects, both cyst passage and coproantigen levels were reduced by ~50% in those taking WG compared with the placebo group (P < 0.01 and P = 0.06, respectively). In symptomatic subjects, cyst passage and coproantigen levels fell precipitously in response to metronidazole therapy, and there were no clinically important differences between those receiving supplemental WG or placebo. However, symptoms appear to have resolved more rapidly in the subjects taking WG in addition to metronidazole. The WG supplement was well tolerated in both symptomatic and asymptomatic subjects. These data suggest that components of WG, possibly WGA, either alone or in combination with antiprotozoal agents, can influence the course of human giardiasis.

INTRODUCTION

Giardia lamblia is among the most common protozoan parasites of the human intestinal tract and is a major cause of waterborne enteric disease worldwide.1,2 Giardia infects a wide range of mammalian hosts, including both domestic and wild animals and humans.1,3 Surface water in many regions of the world is heavily contaminated with G. lamblia cysts.4 Upon ingestion, G. lamblia excysts and colonizes the upper gastrointestinal tract, where it can cause a spectrum of disease, from asymptomatic cyst passage to explosive or chronic diarrhea.1,5 Although G. lamblia is not invasive, infection can result in extensive damage to the small bowel mucosa, leading to malabsorption.1,5 Without treatment, people can remain infected for months.1 Many subjects have difficulty tolerating the drugs available for the treatment of giardiasis (e.g., metronidazole), and 10–15% need repeated courses of therapy to be cured.2 In hyperendemic regions of the world, reinfection frequently occurs within a matter of weeks after treatment.6

Lectins are proteins that bind to carbohydrate (sugar) moieties. Lectins were originally recognized because of their ability to agglutinate blood cells’ (hence the term “agglutinins”).7 A wide range of biological actions is mediated by lectin-glycoprotein interactions, including intracellular sorting of proteins, control of morphogenesis, cellular differentiation, fertilization, binding of microorganisms to target tissues, and cell-cell interactions such as adhesion and trafficking of leukocytes, metastasis, and inhibition of natural killer cell activity.7–10 Many lectins are derived from plant seeds,11 and some of the best known are components of common human foods such as kidney beans (Phaseolus vulgaris agglutinin), lima beans (lima bean agglutinin) and wheat (WG agglutinin, or WGA).11 Some dietary lectins are poorly absorbed in the gastrointestinal tract and are relatively nontoxic at moderate concentrations.11 The precise role of lectins in plants is unknown.12,13

The surface proteins of many intestinal protozoa, including G. lamblia, are glycosylated.14,15 At modest concentrations (10–100 µg/mL), WGA can arrest G. lamblia trophozoite growth in axenic cultures,16 inhibit excystation,17 and decrease cyst passage in mice infected with the closely related organism G. muris.16 These observations prompted us to initiate a small clinical trial to evaluate the potential for WGA to influence the course of human giardiasis. In human terms, the doses of WGA used in the studies mentioned above were large but not massive. As much as 10–12% of the protein content of WG is WGA, and common wheat-based cereals contain up to 50 µg of WGA per gram.16 At very high doses, some lectins (including WGA) can have antinutritive effects.11 For this reason, and because our interest in practical solutions for problems in the developing world, we chose to use WG in the current study rather than purified lectin.

MATERIALS AND METHODS

Human subjects. Subjects were recruited at several travel clinics in Montreal (McGill Center for Tropical Diseases, St. Luc Hospital, Maisonneuve Rosemont Hospital) and in public outpatient clinics located in San Juan de Lurigancho, a periurban area of Lima, Peru. Stool samples submitted to the
parasitology laboratories of the participating institutions were screened for the presence of *G. lamblia* by microscopy and coproantigen detection (see below), and people were contacted to determine their willingness to participate in the study. A total of 63 otherwise healthy adults were recruited in Montreal, Canada (*n* = 31), and Lima, Peru (*n* = 32). Subjects who sought consultation in these clinics for any gastrointestinal complaint were considered to be symptomatic (*n* = 38), whereas those for whom stool examination had been performed for other reasons (e.g., posttravel screening, weakness, parasites found in a family member) were classified as asymptomatic (*n* = 25).

Informed consent was obtained from subjects, and human experimentation guidelines of the Medical Research Council of Canada were followed. This study was approved by the ethics review committees of McGill University (Montreal, Canada) and Universidad Peruana Cayetano Heredia (Lima, Peru).

**Treatment protocol.** After obtaining informed consent, subjects were block randomized to receive either WG (2 g) or cornstarch (2 g) orally 3 times a day for 10 days. Block randomization was accomplished by the placement of numbered study packages at the different recruitment sites. Symptomatic patients received simultaneous therapy with metronidazole (250 mg by mouth 3 times a day for 7 days). Asymptomatic subjects received either WG or placebo alone for 10 days, followed by 7 days of metronidazole (as above). Any subject who remained cyst-positive at the end of the 35-day study period was offered repeat metronidazole therapy.

**Preparation of WG and placebo capsules.** Wheat germ and placebo capsules were prepared in the Montreal General Hospital Pharmacy by grinding raw WG (Indigo Natural Foods, Montreal, Canada) and cornstarch into fine powders and loading in opaque gelatin capsules. The capsules containing WG and placebo were indistinguishable.

**Sample collection and handling.** All subjects kept a diary for the first 10 days in which they recorded the presence or absence of each symptom: fever (temperature > 38°C or any of flushing, chills or rigors), nausea, vomiting (≥ 1 episode/24 hr), bloating, diarrhea (≥ 3 loose bowel movements/24 hr), cramping, and fatigue. Each symptom was arbitrarily assigned a value of 1, and a symptom score was calculated daily on the basis of each subject’s self-reporting. Stools were collected daily (Montreal) or every other day (Lima) for the first 10 days and at Day 35. Each 40-mL stool sample was collected directly into 60 mL of sodium acetate formalin fixative and processed within 10 days of collection. A 2-mL aliquot from each specimen was stored at −70°C until used to determine coproantigen levels. Samples collected in Lima were shipped to Montreal for analysis.

**Stool microscopy and coproantigen detection.** Stools were processed for microscopy with formalin-ether concentration and iodine staining as well as hematoxylin permanent staining in the parasitology laboratory of the McGill Center for Tropical Diseases. Each slide was examined for 10 min by a trained technologist blinded to the origin of the specimen. Slides were scored on a scale of 0–4+ (0 = no cysts or trophozoites seen; 0.5+ = 1–5 cysts or trophozoites per slide; 1+ = 6–15 cysts or trophozoites per slide; 2+ = 16–25 cysts or trophozoites per slide; 3+ = 1 cyst or trophozoite per low-power field [×10]; 4+ = 1 cyst/trophozoite per high-power field [×40]). *Giardia lamblia* coproantigen levels were determined by a commercial enzyme-linked immunosorbent assay (LMD GL-35, Alexon-Trend, Ramsey, MN) according to the manufacturer’s instructions. Results are reported as optical density values. Although this kit is sold as a qualitative test, the optical density values are a rough reflection of coproantigen levels over a moderate range of dilutions. At initiation of therapy, the Spearman rank correlation coefficient between microscopic cyst or trophozoite counts and the coproantigen levels was highly significant (*P* < 0.0001).

**Statistical analysis.** The Canadian and Peruvian study sites gave very similar results, and all analyses used pooled data unless otherwise specified. All data were log-transformed to approximate normal distribution. Baseline-adjusted comparisons between groups on individual days were performed by the Mann-Whitney *U*-test. Comparisons between groups over time were performed by repeated measures analysis of variance (ANOVA), controlling for daily fluctuations and by an autoregressive correlation matrix of order 1. Other tests used are indicated. All analyses were performed by either StatView version 5 or SAS version 6.12 software (both SAS, Cary, NC). All results are reported as means ± standard error of mean.

### RESULTS

**Study subjects.** Characteristics of the subjects recruited to the study are summarized in Table 1. Of the 63 people enrolled in the study, one asymptomatic subject recruited in Montreal withdrew because of the inconvenience of collecting samples (WG arm). Among the 38 symptomatic people, 21 were randomized to the WG arm and 17 to the placebo arm. Among the 24 asymptomatic subjects, 11 were randomized to receive WG, and the remaining 13 received placebo.

**Symptomatic giardiasis.** Wheat germ and placebo groups were comparable in terms of age and sex ratios at the time of recruitment (Table 1) as well as cyst or trophozoite scores (2.2 ± 0.3 versus 2.3 ± 0.4, respectively), stool coproantigen levels (optical density, 0.95 ± 0.15 versus 1.05 ± 0.2) and symptom scores (2 ± 0.7 versus 1.6 ± 0.6). All measures of *Giardia* infection declined with the initiation of therapy such that by Days 5–6 of metronidazole treatment (with or without WG), cyst or trophozoite scores were approaching zero in concentrates (0.05 ± 0.05 versus 0 respectively) and hematoxylin preparations (0.25 ± 0.25 versus 0) (Figure 1a, b). Coproantigen levels and symptom scores fell more slowly over the first week of therapy in both groups (Figure 1c, d).
Figure 1. Impact of metronidazole therapy (250 mg by mouth 3 times a day for 7 days) supplemented with either wheat germ or placebo (cornstarch) at 2 g by mouth 3 times a day for 7 days in subjects with symptomatic giardiasis. Samples were collected daily (Canada) or every other day (Peru). Study outcomes were (a) Giardia lamblia cyst or trophozoite numbers in stool concentrates, (b) G. lamblia cyst or trophozoite numbers in hematoxylin-stained stool samples, (c) stool G. lamblia coproantigen level, and (d) self-reported symptom scores. * P < 0.05, ** P < 0.04, *** P < 0.02.

Compared with the placebo group, symptom scores were slightly higher in the WG group at the time of recruitment but fell rapidly such that subjects receiving WG were largely free of symptoms on Days 5–10 (versus symptom scores of 0.5–1.2 in the placebo group). At Day 35, the subjects in both groups were comparable for all parameters, and only 2 of 21 (WG) and 1 of 17 (placebo) had microscopic evidence of G. lamblia infection (not significant by chi-square analysis). Two subjects with persistently cyst-positive stools despite treatment were identified in Canada and one in Peru.

Asymptomatic cyst passage. Asymptomatic patients who passed cysts assigned to the WG and placebo arms of the study were also comparable for age and sex ratios (Table 1), as well as for all study parameters at the time of randomization (Figure 2). Although there was considerable individual day-to-day variability, mean cyst or trophozoite counts...
FIGURE 2. Impact of either wheat germ (WGA) or placebo (cornstarch) at 2 g by mouth 3 times a day for 7 days in subjects with asymptomatic *Giardia lamblia* cyst passage. All subjects received metronidazole therapy (250 mg by mouth 3 times a day for 7 days) beginning on Day 10. Samples were collected daily (Canada) or every other day (Peru). Study outcomes were (a) *G. lamblia* cyst or trophozoite numbers in stool concentrates, (b) *G. lamblia* cyst or trophozoite numbers in hematoxylin-stained stool samples, (c) stool *G. lamblia* coproantigen level, and (d) self-reported symptom scores. *P < 0.03, **P < 0.02.

(by concentrates or hematoxylin stain) and coproantigen levels were stable over the initial 10-day period of observation in the placebo group. Compared with the placebo group, cyst or trophozoite numbers in stool concentrates were rapidly reduced (~ 60%) in those receiving WGA (2.2 ± 0.4 versus 0.9 ± 0.4; P < 0.04 on Day 2 of study). Baseline-adjusted differences in concentrate cyst or trophozoite counts between placebo and WGA groups reached significance (P < 0.05) on several individual study days (Figure 2a), and this effect persisted for the 10-day study period (repeated-measures ANOVA P < 0.01).

Similar reductions in scores for the WG group were evident in hematoxylin-stained slides and coproantigen levels, but differences between placebo and WG groups took 3–4 days to become apparent and the confidence intervals were somewhat wider. As a result, only borderline significance was achieved for these parameters on individual days and by repeated-measures ANOVA (hematoxylin P = 0.14 and...
coproantigen $P = 0.06$; Figure 2b, c). It is noteworthy that subjects in this arm of the study described themselves as asymptomatic at recruitment, yet more than half recorded one or more symptoms compatible with Giardia infection in their diaries on the first day of placebo or WG treatment. There was wide day-to-day variability in the symptoms reported by these subjects and no significant differences between the placebo and WG groups (Figure 2d).

By Day 35 (−2 weeks after completion of metronidazole therapy), there were no significant differences between the placebo and WG groups for any parameter. At Day 35, 5 of the 22 stool samples available from initially asymptomatic subjects were positive for cysts or trophozoites (2 of 9, 22%, in the WG arm and 3 of 13, 23%, in the placebo arm, 2 in Canada, 3 in Peru). As was the case for the symptomatic subjects, we do not know if these results reflect treatment failures or reinfection. These 5 subjects remained asymptomatic but were offered retreatment with metronidazole nonetheless.

**DISCUSSION**

The results of this small, placebo-controlled, double-blind study indicate that some component of ingested WG (possibly WGA) can act against G. lamblia in the human gastrointestinal lumen. An anti-infective role for lectins has long been suspected because of their polyvalent structure and their conservation and distribution in nature. Although this potential was recognized soon after the discovery of lectins, the perceived nonspecificity of lectin binding and the subsequent discovery of antibodies limited interest in these molecules for at least 2–3 decades. Early descriptive work that used lectins to study G. lamblia glycosylation patterns found the exclusive use of N-acetyl-glucosamine (GlcNAc) moieties by these parasites. Ortega-Barria and others subsequently demonstrated that WGA (which has specificity for GlcNAc) could induce reversible growth inhibition of G. lamblia trophozoites in axenic culture and halve cyst passage in mice infected with the closely related species, G. muris. Meng and others simultaneously demonstrated that WGA could inhibit G. lamblia excystation as effectively as monoclonal antibodies directed against cyst wall antigens.

We have now built on these observations to demonstrate reduced cyst passage and coproantigen levels in human subjects with asymptomatic G. lamblia infection who received modest doses of WG. The response to supplemental WG in these subjects was similar for all of the G. lamblia parameters measured (e.g., stool cyst count by concentrate and hematoxylin staining and stool coproantigen level). The effects of supplemental WG were rapid (differences detectable within 1–3 days for the various parameters) and remained stable over the 10-day period of observation before metronidazole therapy. Although stool volume was not measured in this study, it seems unlikely that the nondigestible fiber content of 6 g of WG could have increased stool bulk sufficiently to decrease cyst or trophozoite counts by 50–60%.

Furthermore, none of the asymptomatic subjects receiving WG noted any change in either stool bulk or consistency. Although WGA itself is poorly digestible (an attractive feature for a potential luminal antiparasitic agent), the effects we observed were not the result of interference with the assays used because both cysts and coproantigen were readily detected in stool samples spiked with purified WGA to a maximum concentration of 20 μg/mL (data not shown). In symptomatic subjects treated simultaneously with metronidazole, WG therapy had no significant impact on either cyst passage or coproantigen levels. However, the power of this small study to detect any such differences was modest. Similarly, the tantalizing appearance in post hoc analyses of WG-accelerated symptom relief in these subjects may have occurred by chance alone. Finally, our data cannot distinguish between potential actions of WGA or other components of WG. Similarly, this study cannot determine whether WG was acting at the level of the parasite, the gastrointestinal epithelium, or both. A large-scale study is currently under way to determine the potential role for WG either alone or in combination with antibiotics (e.g., metronidazole and tinidazole) in subjects with symptomatic giardiasis.

Although G. lamblia can infect a wide range of mammalian hosts, humans are likely to be the primary reservoirs for this organism in many regions of the developing world (e.g., periurban shanty towns). In such settings, the asymptomatic passage of G. lamblia cysts is rarely cause for treatment on simple economic grounds. Because cyst passage can persist for months in untreated subjects, these people likely contribute significantly to household as well as community contamination. This is particularly true because the prevalence of G. lamblia infection is highest in those least likely to be fussy about hygiene (infants 1–3 years old). An inexpensive intervention that reduces cyst passage in these subjects could plausibly have significant impact on environmental contamination and G. lamblia transmission.

Further studies are needed to determine the optimal dose of WG needed to influence G. lamblia cyst passage and to explore the long-term impact of short-term WG therapy on cyst passage, progression to symptomatic illness, and reacquisition of G. lamblia in hyperendemic areas. Limited data suggest that intestinal M cells have particular glycosylation patterns and that lectins can, under certain circumstances, act as immune adjuvants (possibly by protecting antigens from digestion or enhancing antigen delivery to M cells). As a result, future experiments will need to address the question of whether or not WG has the potential to influence the immune response to G. lamblia infection.

As noted above, a wide range of lectins are present in staple foods such as beans and grains. At very high concentrations, several of these lectins have antinutritive effects, with altered gastrointestinal morphology and malabsorption. The precise dose of WGA delivered in the current study was unknown but may have been as high as ~60 mg per day. Although Lorenzsonn and Olsen have reported that even larger doses of purified WGA (200 mg per day) can be ingested for short periods of time without ill effect, a detailed evaluation of possible malabsorption was not performed. In our study, the asymptomatic subjects receiving WG reported slightly more symptoms than those receiving placebo, but these differences did not reach statistical significance. Larger and more detailed studies will be needed to fully determine the safety of lectin administration in patients with human giardiasis.

The current study raises the possibility that other gastro-
intestinal protozoa might also be successfully targeted by lectins, either alone or as adjuncts to standard therapy. Several characteristics of these organisms make such a strategy both plausible and attractive. Like G. lamblia, the surface proteins of other gastrointestinal protozoa are glycosylated.\textsuperscript{25-27} With the exception of Entamoeba histolytica, Balantidium coli, and some Microsporidium spp., the pathogenic intestinal protozoa are either restricted to the gut lumen (e.g., G. lamblia and Dientamoeba fragilis) or reside in gut epithelial cells and reenter the gut lumen to infect new cells (e.g., Cryptosporidium parvum, Microsporidium spp., Isospora belli, and Cyclospora cayetanensis).\textsuperscript{2,26,27} The treatment options available for several of these organisms are limited at the current time (e.g., C. parvum, D. fragilis, and Microsporidium spp. in patients with human immunodeficiency virus or acquired immune deficiency syndrome).\textsuperscript{26-28}

Although our data do not imply that even G. lamblia can be cured by WG alone, many of the common protozoal infections (including giardiasis) have variable clinical presentations.\textsuperscript{1,2} As a result, even modest lectin-induced changes in parasite biology may be sufficient to alter the balance between the immune system and the parasite such that new exposures (or chronic infections in immunocompromised subjects) become either asymptomatic or less symptomatic.

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